Functional Morphology of the Anterior Pituitary Gland. I. The Cell Types and Distribution in the Anterior Pituitary Gland of Pigs of Different Sexes and Ages

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Functional Morphology of the Anterior Pituitary Gland.

I. The Cell Types and Distribution in the Anterior Pituitary Gland of Pigs of Different Sexes and Ages

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Summary

The cells of the pig anterior pituitary gland were classified into seven types by light microscopy, using the authors' specific staining method. By this method, the two types of gonadotrophs were identified.

In 6-months-old pigs, the cell counts of each type showed similar values in the males and females. In the castrated males, a number of the enlarged cells caused by castration were observed, though the so-called "signet ring" castration cell in the castrated rat was not observed. In the castrated pigs, functional changes of LH and FSH cells were exhibited by the reduction of their granular contents and by their hypertrophy.

In 1-month-old pig, the zona tuberalis was already recognized, but the cells in this area were immature and it was difficult to identify the cell types. In 2-months-old pig, the zona tuberalis was more extensive and contained numerous TSH, LH and FSH cells which were fully granulated. The percentages of these cell types were nearly the same as those in 6-months-old pigs.

A number of prolactin cells were observed in 1-month-old pig. The percentage of this cell type did not exhibit any sex difference in 6-months-old pigs.

The cell types of the anterior pituitary gland have been studied in various animals by both light and electron microscopy. In the human anterior pituitary gland, Ezrin (1) classified light-microscopically the gland cells into seven types, employing different staining methods. The researches with electron microscopy have established the morphological criteria for the identification of the cell types (2, 3, 4, 5, 6, 7). These criteria have showed a good correlation with the staining properties of the cell types obtained with light microscopy (7). Now it is generally admitted that the seven types exist in the anterior pituitary gland in various animals.

The cell types of the anterior pituitary gland in the pig have been studied by several authors (8, 9, 10, 11). Shino classified them into six cell types with light microscopy, but failed to distinguish delta 1 and delta 2 cells (8). Mikami and Muto (11) also identified six distinctive types by morphological criteria with electron microscopy.

The aim of the present study is to identify the seven types by light microscopy in the anterior pituitary gland of the pig. The relationship of the sex and age of the animals to the occurrence and distribution of these cell types thus identified was also investigated.

The present study was performed as the first step of a series of the morphological studies on the anterior pituitary gland, with special reference to the secretory activity of the cell types and their morphological criteria.

Materials and Methods

Eighteen Yorkshire/Landrace Hybrid pigs were used in the present study. Twelve of them were six months old. The pituitary glands of those animals were obtained at a slaughter house. They consisted of four each of males, females and castrated males. The castration was performed within one month after birth by the conventional techniques. Six others were females which were obtained from a farmhouse and were one, two, three, four, five and six months old, respectively. The anterior pituitary glands of these animals were removed immediately after slaughter, and were cut into two halves at the median plane. One half was fixed for 8–10 hours in Zenker-formalin mixtures, while the other was fixed for 12–18 hours in Carnoy's fluid. The glands were then dehydrated in ascending grade of alcohol, cleared in xylol and embedded in paraffin routinely. The section fixed in Zenker-formalin mixtures were stained by authors' specific method as follows:

1. Chromation in 5% potassium dichromate for 18–24 hours at 37°C in darkness.
2. Rinse in two changes of distilled water.
3. Stain with aldehyde thionin technique of Paget and Eccleston (12).
4. Wash in running water for 10 minutes.
5. Stain with PAS technique of McManus (13).
6. Wash in running water for 10 minutes.
7. Post-chromation in 5% potassium dichromate for 18–24 hours at 37°C in darkness.
8. Quickly rinse in two changes of distilled water.
9. Stain with authors' method which modified erythrosin-orange G-aniline blue method of Cleaveland and Wolfe (14).
   i) Stain in 0.5% aqueous solution of erythrosin for 45–60 seconds.
   ii) Rinse in two changes of distilled water.
   iii) Stain in 2% solution of orange G dissolved in 1% aqueous solution of phosphomolybdic acid for 2–3 minutes.

iv) Immerse in 1% aqueous of phosphomolybdic acid for 30 seconds.

v) Stain in 1% aqueous solution of aniline blue for 30–40 seconds.

10. Quickly rinse in distilled water, dehydrate in 95% and absolute alcohol, clear in xylol, and mount with permount.

The sections fixed in Carnoy's fluid were stained by the method which combined a dialized-iron-staining method with PAS technic of Ezrin and Murray (15).

Counts of the gland cells were determined by the simplified method (16) of Rassmussen and Herrick (17), and Inoue (18).

Results

1. *The Cell Types and Their Distribution.*

By the authors' specific staining method, the cells of the pig anterior pituitary gland were classified into the seven types (Table 1). Alpha cell (STH cell) was stained to reddish orange (Fig. 1), epsilon cell (prolactin cell) to distinct yellow (Fig. 2), beta and delta 1 cell (TSH and LH cell) to blue purple (Figs. 3, 4, 5, 6) delta 2 cell (FSH cell) to red purple (Figs. 7, 8), and zeta cell (ACTH cell) to light reddish purple or light blue (Fig. 9). Gamma cell (follicular cell) was stained, if any, weakly to light blue. The staining properties of TSH cell and LH cell were similar. However, it was possible to distinguish them, because the former was irregular or polygonal in shape and was limited to the zona tuberalis, while the latter was oval or round in shape, larger than the former and distributed throughout the pars distalis. This was confirmed by the method which combined a dialized-iron-staining method and PAS technic, by which TSH and FSH cells were stained to red purple, and LH cell to blue purple (Figs. 10, 11).

No characteristic distribution of STH and prolactin cells was observed. LH and FSH cells were distributed throughout the pars distalis, but mainly concentrated in the zona tuberalis. ACTH cells were abundant in the central area of the pars distalis including the zona tuberalis. The distribution of TSH cells was

TABLE 1. *Staining Properties of the Different Cell Types in the Anterior Pituitary Gland of the Pig.*

Cell types	AT-PAS-Erythrosin-Orange G-Aniline blue stain*	Iron-PAS stain**
Alpha cell (STH cell)	reddish orange	—
Epsilon cell (Prolactin cell)	distinct yellow	—
Beta cell (TSH cell)	blue purple	red purple
Delta 1 cell (LH cell)	blue purple	blue purple
Delta 2 cell (FSH cell)	red purple	red purple
Zeta cell (ACTH cell)	light red purple or light blue	—
Gamma cell (Follicular cell)	light blue or not stain	—

* Authors' specific staining method which combine the different staining methods.

** The method which combined a dialized-iron-staining method with PAS technic (15).

limited to the zona tuberalis. However, cells of this type were very rare in other parts of the pars distalis.

2. *Zona Tuberalis.*

In the pars distalis of the pig, as Shino reported (10), the zona tuberalis was recognized as the area which was especially abundant in TSH, LH and FSH cells, but contained few STH and prolactin cells. The distribution of those cell types in the area was similar to that of the zona tuberalis in the bovine and sheep pituitary, reported by Mikami (7), and Mikami and Daimon (19).

The zona tuberalis of the pig was larger in the females and the castrated males than in the males. In 1-month-old pig, the zona tuberalis was indistinct and contained numerous immature, non-granulated cells. Few cells possessed a small number of granules. In 3-months-old pig, the zona tuberallis was more extensive and contained a number of mature, fully granulated cells.

3. *The Relationship between Sexes, and Ages, and the Percentages of the Cell Types.*

Cell counts on the anterior pituitary gland of 6-months-old pigs showed similar values in the males and the females. In the zona tuberalis of the castrated males, the number of the enlarged gonadotrophs (Figs. 12, 13) and the enlarged aniline blue-positive cells (Figs. 14, 15) increased, while that of follicular cells decreased (Table 2). The enlarged aniline blue positive cells were classified into two types according to the difference of the staining properties. One of them had a cytoplasm which was diffusely and very weakly stained with aniline blue. It contained the distinct Golgi apparatus and a few granules of blue purple (Fig. 16) or red purple (Fig. 17). The other was stained darker than the former, the Golgi apparatus of which was not evident (Fig. 18). It occasionally contained a condensed or pyknotic nucleus. The percentages of these two cell types were different in the castrated pigs.

In 4-month-old pig, all the cell types were observed. In 2-months old one, TSH, LH and FSH cells increased in number, and in 3-months-old one, the percentages

TABLE 2. *Relative Percentages of the Different Cell Types in the Anterior Pituitary Gland of the 6-months-old Pigs.*

Cell types	male	female	castrated male
Alpha cell (STH cell)	22-28%	20-25%	19-23%
Epsilon cell (prolactin cell)	13-19%	14-20%	12-17%
Beta cell (TSH cell)	5- 9%	4- 8%	5- 8%
Delta 1 cell (LH cell)	6-10%	7-11%	7-14%
Delta 2 cell (FSH cell)	8-12%	9-14%	11-15%
Zeta cell (ACTH cell)	4- 7%	3- 8%	4- 8%
Gamma cell (Follicular cell)	30-38%	31-36%	22-29%
Aniline blue positive cell*	1- 3%	2- 4%	7-13%

* The enlarged aniline blue positive cells caused by castration.

of the identified cells were very similar to those of 6-months-old pigs.

A number of prolactin cells were observed in 1-month-old pig. The percentage of this type did not exhibit any difference between the males and the females of six months old.

Discussion

In the present study, the cells of the pig anterior pituitary gland were classified into seven types, which corresponded to those in the human pituitary by Ezrin (1), in the bovine pituitary by Mikami (7), in the sheep pituitary by Mikami and Daimon (19), in the pig pituitary by Shino (10), and Mikami and Muto (11), with respect to the staining properties and morphological characters. In the pig anterior pituitary gland, delta 1 and 2 cell which were not identified so far with light microscopy could be distinctly identified, using authors' specific staining method and the combination of a dialized-iron-staining method with PAS technic, as reported by Ezrin and Murray (15).

The zona tuberalis is well developed in the bovine (7, 20, 21), goat (22) and sheep (19) anterior pituitary gland. Shino adopted the term medulla in the bovine anterior pituitary (20). He revealed that the medulla can be already observed at birth, and is abundant in blood vesseles and connective tissue, and that the beta and delta cells in the area are small and not evident. In 150-days-old bovine anterior pituitary glands, the area is more extensive and contains numerous beta and delta cells which are well developed. According to Mikami and Muto (4), and Mikami and Daimon (19), the thyroidectomy cells especially concentrate to the zona tuberalis in the anterior pituitary gland.

In the anterior pituitary gland of 1-month-old pig, the zona tuberalis was not well developed. In the 3- and 6-months-old one, the area was fully developed. In the castrated pigs, the area was more extensive and the fully developed, granulated cells were numerously observed.

From the evidence previously described, the zona tuberalis is considered to develop fully as early as in three months after birth. According to Ezrin (1), the area comparable to zona tuberalis in the human anterior pituitary gland is mainly supplied the blood of long hypophysial portal veins. Therefore, the zona tuberalis is considered to be intimately related with the hypothalamus and may have some specific functions which could not be revealed in the present study.

In the castrated pigs, the so-called "signet ring" castration cells in the castrated rat (15, 23) were not observed as reported by Kato (8) and Shino (10). However, the enlarged cells which were two times as large as those in the normal males were recognized. These cells were stained with PAS and/or aniline blue. The cells which were stained by aniline blue were classified into two types. According to the conception on the secretory cells of Ham (24) and Kurozumi (25), the secretory cells degranulate when its function was activated and elevated. It is,

therefore, considered that the cells which were very weakly stained with aniline blue, and had a few blue purple granules and the distinct Golgi apparatus were the degranulated condition and the active stage of hormone secretion. The cells which were stained darker than the former with aniline blue, and also stained with PAS technic had been already declined in the secretory function and were going to degenerate.

It appeared that the cells mentioned above were gonadotrophs caused by castration, because they were hypertrophic and concentrated in zona tuberalis. It seemed that the enlarged cells which were positive with PAS technic were FSH cells, and those which stained very weakly with aniline blue and contained a few blue purple granules and the distinct Golgi apparatus were LH cells. The enlarged cells which stained by aniline blue and PAS technic appeared to be FSH and/or LH cells which have been already declined in hormone secretion and fallen in degeneration. The counts of these cell types showed apparent individual variation in the castrated pigs. This suggested that the reactivity of hypothalamus for castration was individually different in young pigs.

In the present study, prolactin cells were numerously observed in 1-month-old pig, in which the mammary glands were not developed. The percentage of this type was also not different among the three sex groups of 6-months-old pigs. This suggested that either prolactin cells secrete some hormone in addition to prolactin as discussed by Shino (10), or prolactin possesses an effect which has been unknown as discussed by Gropp (26).

In the present study, the classification of the anterior pituitary gland cells by Ezrin[1)] was applied. Nakane[27)], however, showed that follicle stimulating hormone and luteinizing hormone were frequently found in the same cell. This suggested that gonadotrophs should not be identified as LH cells and FSH cells by their ultrastructural characteristics.

The present authors think that it is doubtful whether the conclusion of Nakane may be adopted or not for the classification of the cells in the pig anterior pituitary gland. A further evaluation of the classification by immunoenzyme histochemistry is worthy of consideration.

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Explanation of Figures

All photomicrographs except Figs. 10 and 11 were taken from the sections of the anterior pituitary gland of 6-months-old pigs, stained with authors' specific staining method. In Figs. 10 and 11, the sections were stained with the combined method of a dialized-iron staining and PAS technic.

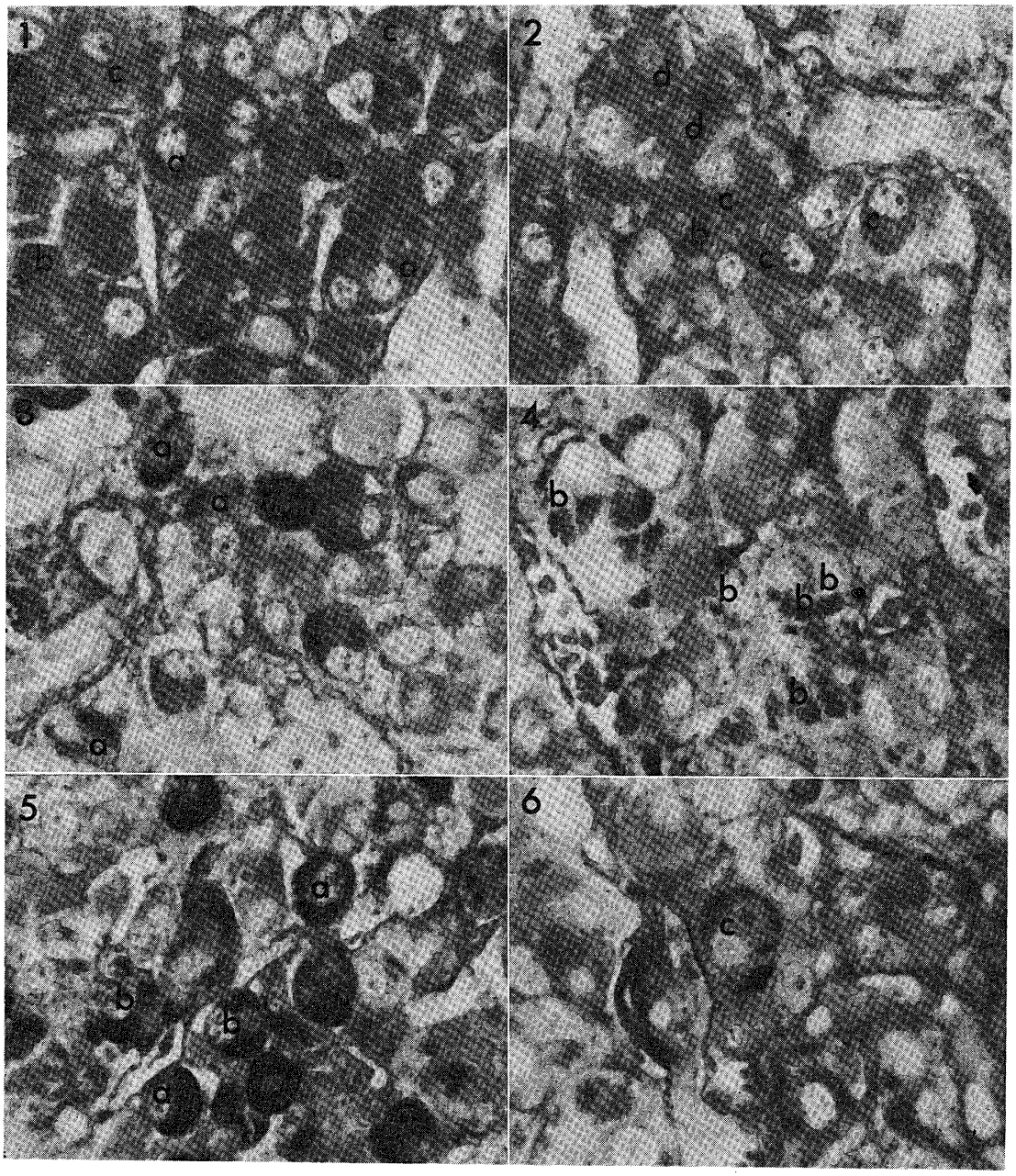
Plate 1

Explanation of Figures

FIGS. 1–2. a: the large alpha cells (STH cells) with a distinct Golgi apparatus. b: the small alpha cells stained darker than the large alpha cells. c: the large epsilon cells (prolactin cells) with a distinct Golgi apparatus. d: the medium epsilon cells slightly stained darker than the large epsilon cells. Male. ×1000.

FIGS. 3–4. a: the beta cells (TSH cells) with a distinct Golgi apparatus. b: the degranulated beta cells. Female ×1000.

FIGS. 5–6. a: the delta 1 cells (LH cells) with a large number of granules. b: the delta 1 cells with a distinct Golgi apparatus. c: the enlarged, degranulated delta 1 cells. Female. ×1000.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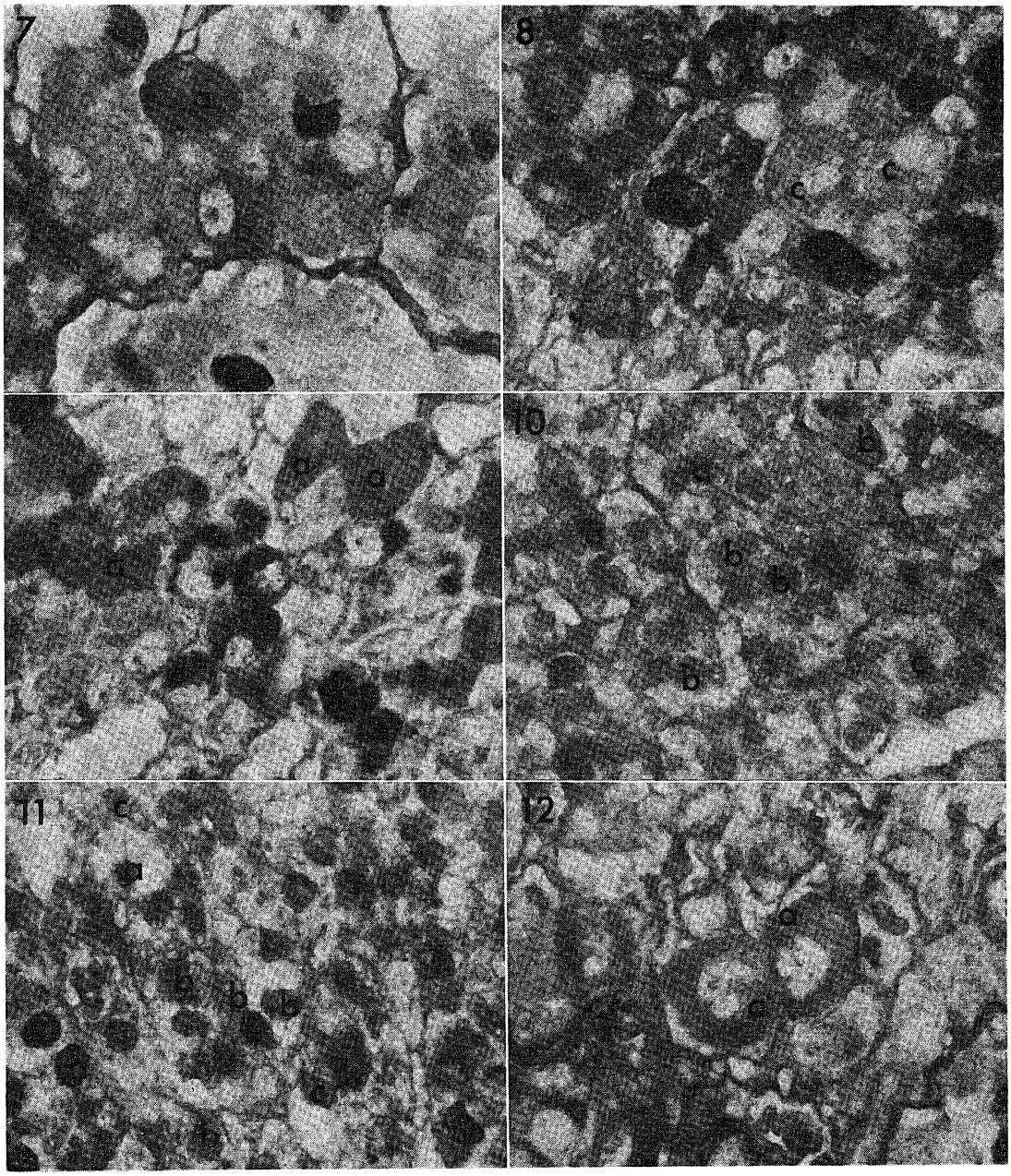
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Plate 2

Explanation of Figures

FIGS. 7–8. a: delta 2 cells (FSH cells) filled with granules. b: the delta 2 cells with a distinct Golgi apparatus. c: the degranulated delta 2 cells. Female. ×1000.

FIGS. 9. a: zeta cells (ACTH cells) stained to light red purple with PAS technic. Male. ×1000.

FIG. 10–11. a: delta 1 cells. b: delta 2 cells. c: beta cells. Delta 1 cells were smaller and stained darker than delta 2 cells. Female. ×400.

FIG. 12. a: the enlarged delta 2 cells caused by castration. Castrated male. ×1000.

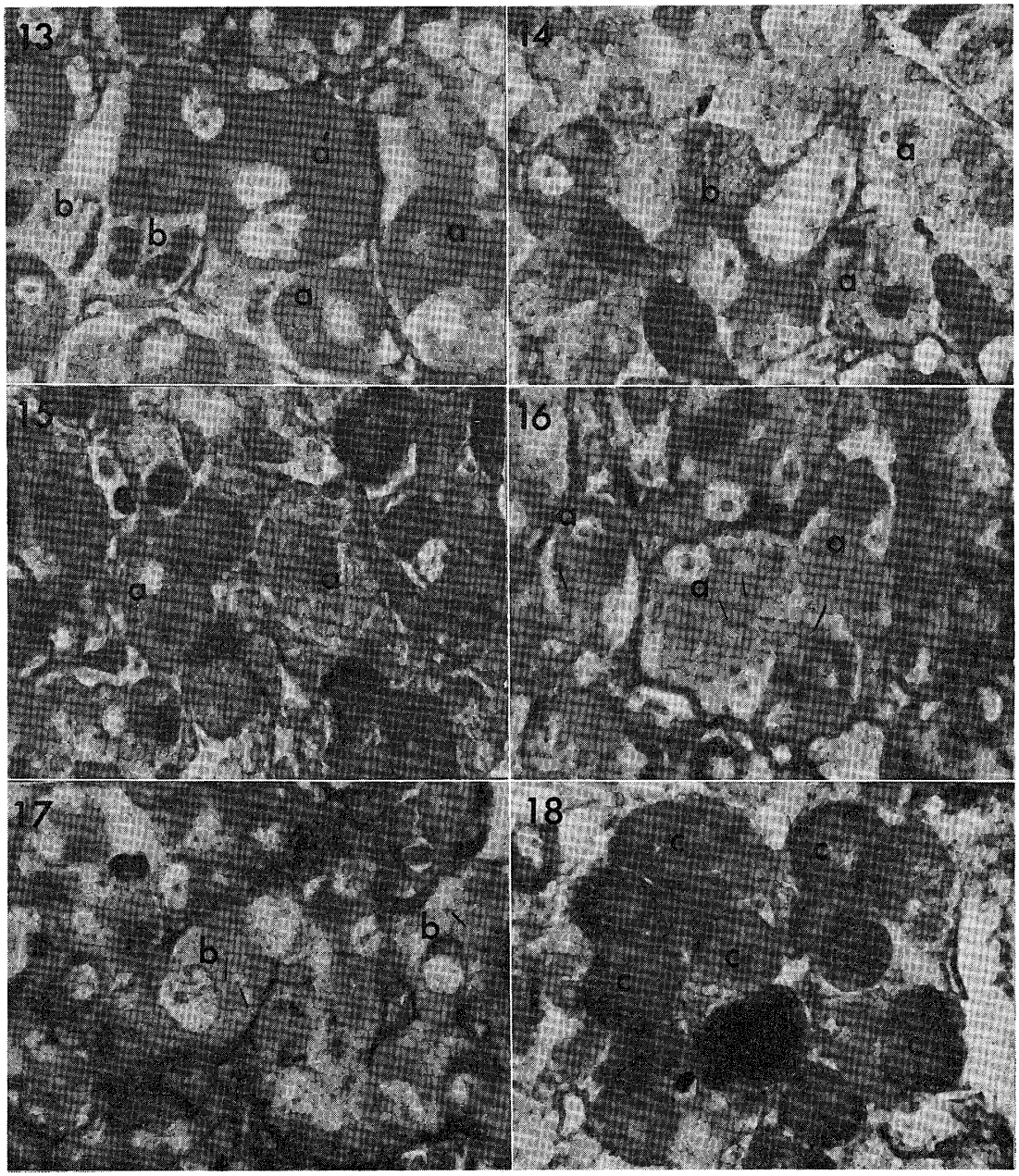
Plate 3

Explanation of Figures

FIG. 13. a: the enlarged delta 2 cells caused by castration. a′: the very large delta 2 cells frequently observed. b: the enlarged delta 1 cells caused by castration. Castrated pig. ×1000.

FIGS. 14–15. a: the enlarged aniline blue positive cells with a distinct Golgi apparatus and a few granules caused by castration. b: the cells stained darker than the formers with aniline blue. Castrated pig. ×1000.

FIGS. 16–18. a: the enlarged aniline blue positive delta 1 cells with a few blue purple granules (/). b: the enlarged delta 2 cells with a few red purple granules (/). c: a group of the enlarged and degenerated cells observed in castrated pigs. Castrated pig. ×1000.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